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(54) Dietary composition

(57) The composition comprises 0.5 to 50% wt. of selenium-containing alga and 99.5 to 50% wt. of C_{18-22} unsaturated fatty acids containing at least two double bonds or their derivatives.

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DIETARY COMPOSITION AND PROCESS FOR PREPARING SAME

This invention relates to dietary compositions.

In an other aspect of the invention, there is provided a process for preparing these compositions.

It is known that the C_{18-22} ω-3 unsaturated fatty acids possess advantageous biological properties. Amongst these substances, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are outstanding; the importance and multiple biological effects of both acids have been discussed by Dyerberg et al. [The Lancet 15, 117 (1978)].

The effects, connected with the important role of the polyunsaturated fatty acids, mainly EPA and DHA, in the hyperlipidaemia and thrombotic diseases have been reviewed by Goodnight et al. [Arteriosclerosis 2, 87 (1982)].

The active components of the fish-oil, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are precursors of the biosynthesis of the PG-3 series and simultaneously they are competitive inhibitors of harmful metabolites such as TXA_2 and TXB_2 arising from the so-called "arachidonic acid cascade" which is a chain of complicated biochemical processes starting from arachidonic acid.

In addition to many preferably effects, a drawback of the polyunsaturated fatty acids consists in the fact that they represent a possible source of the most harmful malondialdehyde (MDA) resulting from radical peroxidation processes whereby the so-called "ceroidal lipofuscinosis" can be induced in the central nervous system.

The utilization of EPA for alimentary purposes in the form of a cyclodextrin inclusion complex has been described in the French patent specification No. 2,550,445. Marschall et al. [Am. J. Clin. Nutr. 38, 895 (1983)] studied the role of ω-3 polyunsaturated fatty acids in the synthesis of α-linolenic acid and prostaglandins as well as in the dietary alimentation.

In addition to EPA and DHA, the fish-oils contain high amounts of saturated and lowly saturated fatty acids as well as nonhydrolyzable components the removal of which is very important since the triglyceride level of blood and the calory intake are sensitively enhanced by the increase in the dietary dose. Furthermore, stercids which are harmful from a dietetic point of view, e.g. cholesterol as well as vitamin D (its precursor) and vitamin A, both liable to accumulate in the human organism, can also be present among the nonhydrolyzable constituents.

The dietary effects of the ω-3 polyunsaturated fatty acids and mainly of EPA and DHA in relation to their manifold physiological connections are discussed in a high number of other papers [see e.g. U. Barcelli: Thromb. Res. 39, 307 (1985); J.J. Jurkowski: JNCI 74, 1145 (1985); A. Lembke: Milchwissenschaft 40, 329 (1985)].

It is also known that algae have from time immemorial been used by the mankind for the purpose of nutrition and feeding. Thus, algae are mainly consumed by the peoples of the Far East; recently, however, they are utilized in dry form or in the form of tablets in the developed countries, to-

Algae are the carriers of highly valuable nutritive materials since their dried form contains in high concentration substances which are essential for a healthy life such as vitamins, proteins, complexes of proteins with micro-elements, saccharides, polyunsaturated fatty acids and the like.

A general and detailed review on algae is found e.g. in the book of Zajic: "Properties and Products of Algae" (Edition Planum, New York, 1970).

The investigation on the biological effects of micro-elements and trace elements has been started in the last decades. Thus, it has been recognized that selenium is one of the most important, essential substance of life. The preferable action of selenium mainly lies in the activation of the glutathione-peroxidase enzyme, more precisely in the activation of the prosthetic group of the enzyme which is the most important endogenic inhibitor of the damaging peroxidation processes. Selenium is not accumulated in the human organism; thus, it has continuously to be supplemented. Up to the present, selenium has been supplied in the form of inorganic compounds, mainly as selenium dioxide or sodium selenite.

Selenium in itself has hypotensive effect, improves the ischaemic, hypoxic and infarction states of the heart and inhibits the ceroidal lipofuscinosis of the central nervous system; it also exerts a beneficial effect on periodontitis and proved to diminish the probability of the development of cancer diseases; furthermore, it is considered to be a

mutagenesis-inhibiting agent. A number of alterations or diseases, respectively, such as liver necrosis, myonecrosis, destruction of the erythrocyte membrane, interstitial laesions, ST-elevation in the ECG, kwashiorkor syndrome (protein malnutrition) and multiplex sclerosis proved to be induced by selenium deficiency.

A review on the biological effects of selenium was published by Thressa et al. [Nutrition Review 35, 7 (1977)], R. I. Shamberger [J. of Env. Path. and Tox. 4, 305 (1980)]; as well as Masukawa et al. [Experientia 39, 405 (1983)].

The aim of the present invention is to develop a dietary composition, which eliminates the disadvantageous biological effects of the consumption of polyunsaturated fatty acids, provides the combination of the advantageous properties of EPA, DHA, algae and selenium, and is capable, on the basis of its composition, to counterbalance at least a part of unhealthy life conduct and disadvantageous habits of alimentation.

The invention is based on the recognition that the above aims can completely be achieved by a food composition which comprises a selenium-containing alga together with polyunsaturated fatty acids.

Thus, the present invention relates to dietary compositions, which comprise 0.5 to 50% by mass of selenium-containing alga as well as 99.5 to 50% by mass of C_{18-22} unsaturated fatty acids containing at least two double bonds or their derivatives together with additives commonly used in the food industry and optionally with an antioxidant.

Furthermore the invention relates to a process for

preparing the above dietary composition, which comprises mixing 0.5 to 50% by mass of selenium-containing alga as well as 99.5 to 50% by mass of C_{18-22} unsaturated fatty acids containing at least two double bonds or their derivatives, with additives commonly used in the food industry and optionally with an antioxidant.

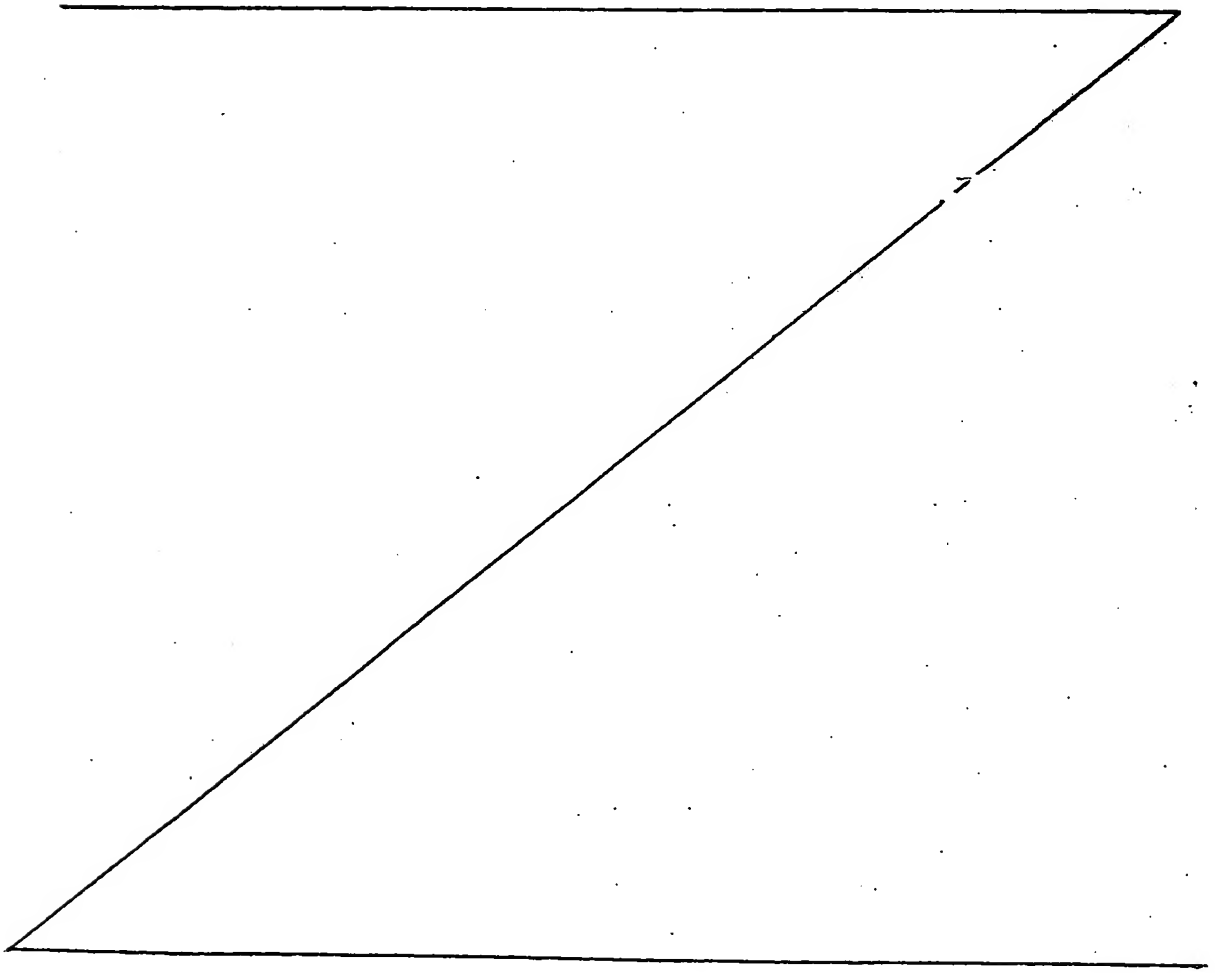
The selenium-containing algae can be prepared by cultivating algae on or in an aqueous medium containing 10^{-7} to 2×10^{-3} moles per litre of a soluble organic and/or inorganic selenium compound; preferably the algae used for cultivation is selected by treating a strain of algae with N-methyl-N'-nitro-N-nitrosoguanidine and propagating the strain on a selenium-containing propagation medium and selecting for further cultivation those individuals that both incorporate selenium readily and have a high growth rate. Preferred algae species include unicellular blue or green algae, e.g. Chlorella sp., Scenedesmus sp., or Spirulina sp., and in particular Aphanocapsa thermalis, Chlorella minitissima, Chlorella fusca, Scenedesmus obtisiusculus, Scenedesmus obliquus or Nostoc commune (as described in our co-pending application corresponding to HU 575/88). In this way, selenium accumulates in the organism of the growing algae.

The raw material of the C_{18-22} ω-3 unsaturated fatty acids, serving as active ingredients of the composition, may be the oils obtained from various marine or fresh-water fishes, mainly the oils from mackerel, cod-fish, herring, sardine and ink-fish as well as the oils which may be obtained from the liver of these fishes such as cod-liver oil and shark-liver oil.

As algae Chlorella or Scenedesmus strains are mainly used which are not only useful for human comsumption but also have own advantageous biological action.

The composition is sensitive to oxidation. Thus, it is suitable to use an active preserving agent, e.g. α-tocopherol (vitamin E), glutathione or a traditional anti-oxidant such as butylhydroxytoluene.

The homogenized composition can be included in alimentary products, tablets, capsules and the like. Here, the term "alimentary product" means all useful products that can orally or parenterally be administered. The orally administered product may be contained in a confectionary



or sweets industry article (e.g. chocolates, cakes), meat or butcher industry compositions, spices, margarine, milk, butter or fat products, oils and the like.

The main advantages of the composition according to the invention can be summarized as follows.

a) The individual components of the composition have in itself important biological and alimentation effects and combine the preferable properties of EPA and DHA as well as selenium and algae.

b) It eliminates the possibility of "ceroidal lipofuscinosis" appearing on the consumption of polyunsaturated fatty acids.

c) The use of a continuous dietary regimen is not required by the consumption of the composition according to the invention since it does not contain the harmful fish-oil components discussed above (saturated fatty acids, cholesterol, vitamins A and D).

d) Selenium is present in a natural form enriched in algae, i.e. as a material of native origin.

e) It is useful to prevent apoplectic and thromboembolic states of the cardiovascular system, thus to inhibit the development of infarction and apoplexy as well as to prevent atopically related or atopic disturbances. The use of the composition is highly preferred in developed conditions, too.

The composition of the invention is illustrated in detail by the following non-limiting Examples. The preparation of the components used as basic materials is shown in

Examples 1, 2 and 3 whilst the preparation of the compositions is described in the further Examples.

Example 1

Ten kg of sardine-oil are added at a temperature of 50 to 60 °C to a solution containing 2 kg of sodium hydroxide in 70 litres of 95% ethanol. The mixture is refluxed under nitrogen for 2 hours, then cooled to 10 °C while stirring. The precipitated crystalline sodium salt of the saturated fatty acid is filtered and washed with a little ethanol. The ethanolic filtrate is evaporated and then 20 litres of boiled-out water are added to the residue. The non-hydrolyzable compounds such as cholesterol are removed by extraction with 5 litres of hexane. The extracted aqueous phase is acidified to pH 2 by adding dilute sulfuric acid and it is again extracted with 15 litres of hexane. The organic phase is washed with water, dried over anhydrous sodium sulfate and evaporated to give 3.2 kg of an oily concentrate with a DHA content of 36.8% and EPA content of 31.8%. This oil is brown and smells of fish. Thus, Fuller's earth is mixed to the oily concentrate which is then heated at 105 °C under nitrogen for 10 minutes and filtered as hot. The deodourization is carried out by steam distillation under reduced pressure of 1.3 bar at 170 °C for 3 hours to give 1.6 kg of a light yellow, odourless and tasteless oil with practically unchanged composition.

Example 2

24 kg of cod-liver oil are dissolved in 16 litres of methanol at 60 °C, then 6 kg of 40% sodium hydroxide solution are dropped to this solution at a temperature between 50 °C and 60 °C under stirring. The mixture is stirred at 60 °C for additional 45 minutes. Then, 20 kg of 15% hydrochloric acid are added to the solution at about 60 °C. After separating the phases, the organic layer is extracted with 10 kg of 15% hydrochloric acid and then washed with about 180 litres of hot tap water until neutral. The phases are separated, then 100 litres of acetone are added to the oily phase. The mixture is heated to about 45 °C, then a solution containing 3.8 kg of lithium hydroxide monohydrate in 30 litres of water is added. After stirring for 30 minutes, the mixture is left to stand overnight, then filtered and the acetone filtrate is evaporated. The residue is acidified by adding 8 kg of 15% hydrochloric acid, extracted three times with hexane and then evaporated. The whole operation of purifying is carried out under nitrogen. Thus, 6.4 kg of purified fish-oil are obtained with an iodine number of 258 and an acid number of 160.

One kg of the thus-purified cod-liver oil is dropwise added at 60 °C to the solution of 3 kg of urea in 9 litres of methanol. The mixture is stirred at the same temperature for 2 hours. After cooling down, the mixture is left to stand in a refrigerator at -18°C overnight, then filtered and the filtrate is evaporated. 2.5 litres of hydrochloric acid (concentrated acid diluted to 1:1 with water) are poured

to the residue and the mixture is stirred for 15 minutes. After extracting with hexane, the organic phase is washed with water until neutral, dried over anhydrous sodium sulfate and evaporated to give 0.34 kg of ω-3 fatty acids with an iodine number of 315, EPA content of 24% and DHA content of 42%.

Example 3

Eight litres of Knop-Pringsheim's culture medium are filled into an alga-cultivating glass bottle of 10 litres volume and supplemented with 40 mg of sodium selenite. The system is sterilized at 121 °C under an overpressure of 1 bar for 30 minutes. The sterile solution is inoculated with a pure alga culture of selenium-resistant Scenedesmus obtisiusculus. Sterile air containing 5% by volume of carbon dioxide is bubbled at 25 °C through the culture medium under illumination by an electric discharge lamp working with 4000 lux at a wavelength of 440 to 520 and 640 to 700 /um. After a cultivation period of 14 days the alga is separated from the culture medium. The thus-obtained alga mass is decomposed by supersound and carefully dried at a temperature below 65 °C to give 6 g of an alga powder with a selenium content of 1200 /ug/g.

Example 4

Hundred g of selenium-containing alga (with a selenium concentration of 260 /ug/g of alga powder) are stirred to 150 g of an enriched cod-liver oil of 65% (containing 22%

of EPA and 43% of DHA). After homogenizing, the mixture is preserved by adding 0.2% by mass of vitamin E.

Example 5

Preparation of a dietary margarine composition

Eight g of 65% cod-liver oil (containing 22% of EPA and 43% of DHA) and 1 g of selenium-containing alga (with a selenium concentration of 260 μg/g of alga powder) are mixed to 250 g of soft margarine, then the mixture is homogenized.

Example 6

The process described in Example 4 is followed, except that the following amounts of the starting materials are used:

400 g of 65% enriched cod-liver oil (containing 22% of EPA and 43% of DHA);

70.6 g of alga powder containing 1200 μg/g of selenium; and

0.4 g of vitamin E.

The thus-obtained homogenizate is filled into soft gelatin capsules or soft gelatin bead capsules with a capacity of 500 mg and then included into a blister package.

Example 7

Tablets with the following composition are prepared by using known pharmaceutical devices and process:

Cod-liver oil enriched in EPA and DHA, containing 0.1% of vitamin E as preserving agent	200 mg (DHA content: 43%; EPA content: 22%)
Selenium-containing alga	86 mg (selenium-concentration: 380 µg/g of alga powder)
Lactose	140 mg
Starch	60 mg
Polyvinylpyrrolicone	3.5 mg
Magnesium stearate	3.5 mg

If desired, the tablets are coated with sugar in a panning machine.

Claims

1. A dietary composition, which comprises 0.5 to 50% by mass of selenium-containing alga as well as 99.5 to 50% by mass of C_{18-22} unsaturated fatty acids containing at least two double bonds or their derivatives, in admixture with additives commonly used in the food industry and optionally with an antioxidant.

2. A composition as claimed in claim 1, which comprises esters, alkali earth salts or amine salts as fatty acid derivatives.

3. A composition as claimed in claim 1 or 2, which comprises 5,8,11,14,17-eicosapentaenoic acid and 4,7,10,13,16,19-docosahexaenoic acid as unsaturated fatty acids.

4. A composition as claimed in claim 1, which comprises fatty acids obtained from marine fish-oil as unsaturated fatty acids.

5. A process for the preparation of a dietary composition as claimed in claim 1, which comprises mixing 0.5 to 50% by mass of selenium-containing alga as well as 99.5 to 50% by mass of C_{18-22} unsaturated fatty acids containing at least two double bonds or their derivatives, with additives commonly used in the food industry and optionally with an antioxidant.

6. A dietary composition comprising selenium-containing algae and one or more C_{18-22} unsaturated fatty acids containing at least two double bonds or derivatives thereof, preferably in admixture with edible substances or additives commonly used in the food industry and optionally an antioxidant.

7. A composition as claimed in claim 6, which contains, based on the combined weight of the selenium-containing algae and the C_{18-22} unsaturated fatty acid(s) or derivatives thereof, 0.5 to 50% of the selenium-containing algae and 50 to 99.5% of the C_{18-22} unsaturated fatty acid(s) or derivatives thereof.

8. A process of preparing the dietary composition claimed in claim 6 or claim 7 which comprises admixing the compounds thereof.

9. A dietary composition substantially as hereinbefore described in any one of Examples 4 to 7.

10. A composition as claimed in any one of claims 1, 6 or 7, wherein the fatty acids and/or the algae have been prepared by a process substantially as hereinbefore described in Example 1 or 2 or in Example 3, respectively.

11. A process as claimed in claim 8, substantially as hereinbefore described in any one of Examples 4 to 7.

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